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Respiratory complex I: 'steam engine' of the cell?

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Complex I is the first enzyme of the respiratory chain and plays a central role in cellular energy production. It has been implicated in many human neurodegenerative diseases, as well as in ageing. One of the biggest membrane protein complexes, it is an L-shaped assembly consisting of hydrophilic and membrane domains. Previously, we have determined structures of the hydrophilic domain in several redox states. Last year was marked by fascinating breakthroughs in the understanding of the complete structure. We described the architecture of the membrane domain and of the entire bacterial complex I. X-ray analysis of the larger mitochondrial enzyme has also been published. The core subunits of the bacterial and mitochondrial enzymes have remarkably similar structures. The proposed mechanism of coupling between electron transfer and proton translocation involves long-range conformational changes, coordinated in part by a long α-helix, akin to the coupling rod of a steam engine.

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Introduction

Complex I (NADH:ubiquinone oxidoreductase) is the first enzyme of the respiratory chain in mitochondria and many bacteria. It catalyses the transfer of two electrons from NADH to quinone, coupled to the translocation of four protons (current consensus value [1–3]) across the membrane. In doing so, it provides about 40% of the proton flux during proton-motive force (pmf) generation for the synthesis of ATP [4–9]. Mutations in complex I subunits, including most common pathological mtDNA mutations, have been associated with human neurodegenerative diseases [8,10]. Complex I is a major source of reactive oxygen species (ROS) in mitochondria, which can damage mtDNA and lead to sporadic Parkinson's disease [11] and possibly aging [12]. Mitochondrial complex I consists of 45 subunits (980 kDa combined mass) [13]. The simpler prokaryotic enzyme normally consists of 14 'core' subunits (seven hydrophilic and seven hydrophobic, ~550 kDa combined mass), all conserved from bacteria to humans [4,5,8]. The mitochondrial and bacterial enzymes contain the same redox components (eight to nine iron–sulphur (Fe–S) clusters and flavin mononucleotide (FMN)) and have a similar L-shaped structure [5,14••]. The hydrophobic arm is embedded in the membrane and the hydrophilic peripheral arm protrudes into the mitochondrial matrix or the bacterial cytoplasm [5,8]. High sequence conservation of core subunits indicates that the mechanism is likely to be the same throughout all species, and so the bacterial enzyme represents a 'minimal' model of human mitochondrial complex I.

The hydrophilic domain contains all the known redox cofactors of complex I, involved in electron transfer from NADH to quinone. Understanding how this process is coupled to the translocation of protons across the membrane remains the major question in complex I research [4–6,8]. Two possible mechanisms of coupling have been proposed: 'direct' (redox-driven) and 'indirect' (conformation-driven) [5,6,8,15]. The membrane-spanning part of the enzyme contains the proton translocation machinery but lacks any covalently bound prosthetic groups. This is akin to F-ATPase (which operates by conformational coupling [16]) and is in contrast to cytochrome *c* oxidase (direct coupling involving heme cofactors [17]). The three largest hydrophobic subunits of complex I, NuoL, M and N (*Escherichia coli* nomenclature; subunit names differ between species), are homologous to each other and to Na^+/H^+ antiporter complex (Mrp) subunits [18,19]. They are likely to participate in proton translocation, but reside at a large distance from the hydrophilic domain [20]. A range of cross-linking [21–23] and proteolysis [24] studies suggested conformational changes upon reduction of complex I. All these facts indicate that the coupling mechanism involves long-range conformational changes.

Complex I has for many years resisted attempts to determine its structure and is considered as one of the most difficult membrane protein targets. The paucity of structural data until 2006 has hindered progress in understanding its mechanism. Though the complete atomic structure of this large molecular machine is still unknown, the last two years were marked by major breakthroughs in crystallographic studies of the enzyme, which will be the main subject of current review.

Structures of the hydrophilic domain

Our crystal structure of the *Thermus thermophilus* complex I hydrophilic domain (280 kDa), solved initially at 3.3 Å resolution [25,26] and more recently at 3.1 Å (PDB 3I9V [27••] should be used as a reference), established how eight different subunits are combined to form a continuous electron transfer pathway through the enzyme (Figure 1a). This pathway starts at the tip of the peripheral arm with the primary electron acceptor FMN and extends over ~95 Å through a chain of seven conserved Fe–S clusters to the likely quinone-binding site (Q-site) at the interface with the membrane domain [25,27••] (Figure 1b). The additional subunit Nqo15 stabilizes the complex and is found so far only in species closely related to *T. thermophilus* [8,28]. Its fold is similar to the iron chaperone frataxin and so it may also play a role in the regeneration of proximal Fe–S clusters [27••,28].

We have also solved structures of the hydrophilic domain with NADH bound, in both the fully reduced state (NADH anaerobic; PDB 3IAM) and a partially reduced state (NADH aerobic or dithionite anaerobic) [27••]. The structures explain the kinetic properties of NADH oxidation and ROS production by complex I [27••]. NADH binds in an extended conformation, completely covering the FMN-containing cavity. This means that reduced or semi-reduced FMN (a major source of ROS in mitochondria) can react with solvent and produce ROS only in the absence of bound nucleotide, consistent with functional data [27••,29–32]. The structure thus suggests possible ways to develop a drug to minimize ROS production by complex I [27••].

Binding of NADH and reduction of the complex induce conformational changes involving two areas: around the FMN-containing cavity (adjustments allowing for tight NADH binding) and, importantly, the interface with the membrane domain (Figure 1c). The four-helix bundle of subunit Nqo4/NuoD (*T. thermophilus*/*E. coli* nomenclature) shifts by about 1 Å towards the membrane and helices H1 and H2 from Nqo6/NuoB move 'sideways' (Figure 1c). Since the conformation does not change in the central part of the domain, the likely driving force is the redox state of nearby Fe–S clusters (N2 and N6a/b). Indeed, cluster N2, directly linked to helices H1/H2, is coordinated by the unusual but conserved motif of consecutive (tandem) cysteines, resulting in unfavourable geometry. These cysteines become disconnected upon reduction of the cluster (Figure 1d), leading to backbone shifts, which may provide a unique way to link the redox state of the cluster to protein conformation.

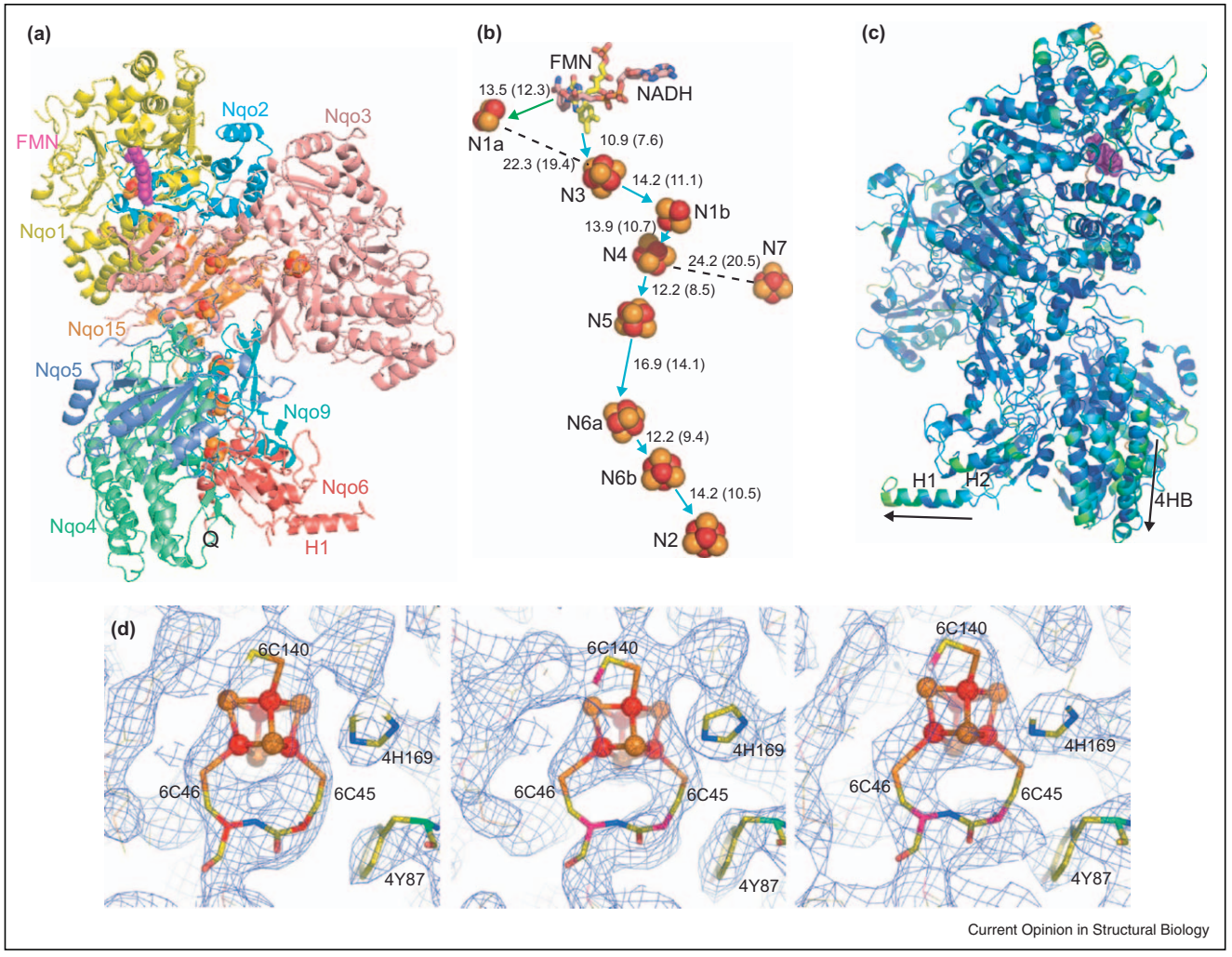
Architecture of the membrane domain and the entire complex

Recently we determined the arrangement of 55 transmembrane (TM) α-helices in the membrane domain of complex I from *E. coli* (Figure 2a) [14••]. Although the limited resolution (3.9 Å) prevented sequence assignment, important mechanistic conclusions could be drawn. First, the structure revealed the arrangement of antiporter-like subunits NuoL/M/N, containing a structurally highly similar core of 14 TM helices (in contrast to previous labelling studies, which suggested 12 helices [19,33]). Second, each antiporter-like subunit contains, in the same positions, two discontinuous TM helices. Such helices are thought to be important for ion translocation in transporters and channels by introducing a charge and flexibility into the membrane [34]. Third, a completely unexpected finding was that the C-terminal extension of subunit NuoL contains a very long amphipathic helix HL, running along almost the entire length of the domain and linking most subunits together. Importantly, HL directly contacts a discontinuous TM helix in each antiporter-like subunit and so is a likely coupling element (Figure 2a). Finally, all antiporter-like subunits are completely separated from the putative Q-site by the belt of smaller subunits (NuoAJK), which excludes direct coupling to electron transfer. A model for helix assignment in antiporter-like subunits has been proposed recently from bioinformatic analyses [35], but it remains to be seen if it is confirmed by crystal structures.

We have also determined the overall architecture of the entire *T. thermophilus* complex I at 4.5 Å resolution (Figure 2b, *E. coli* nomenclature is added as more widely known), using the atomic structure of the hydrophilic domain and the α-helical structure of the *E. coli* membrane domain for molecular replacement [14••] (Supplementary Movie 1). Additionally, we modelled eight TM helices of subunit Nqo8/NuoH, which was absent in *E. coli* crystals. The overall L-shape and dimensions (membrane arm is about 180 Å long) are consistent with most electron microscopy (EM) studies [36–40]. However, the relative position of peripheral arm was unexpected, resulting in a 'sideways' orientation of Nqo6/NuoB helix H1 and placing cluster N2 (electron donor to quinone) about 25 Å away from the expected lipid bilayer surface (Figure 4). Such a large distance implies that the hydrophobic quinone has to move at least 10 Å out of the membrane in order to interact with N2. This is counter-intuitive but may be part of the mechanism, as discussed below.

Complex I can be divided into functional modules evolutionary related to several protein families including hydrogenases and antiporters [41,42] (Figure 2b). For none of the membrane-embedded proteins in these families are the structures known, so we can use complex I as a template to model their architecture (Figure 2c–g). The structure supports and illustrates the hypothesis that parts of independently pre-evolved enzymes gave rise to complex I. Ancestors of current protein complexes consisting of non-overlapping modules (Figure 2c) were likely combined to form a simple proton-translocating

Figure 1



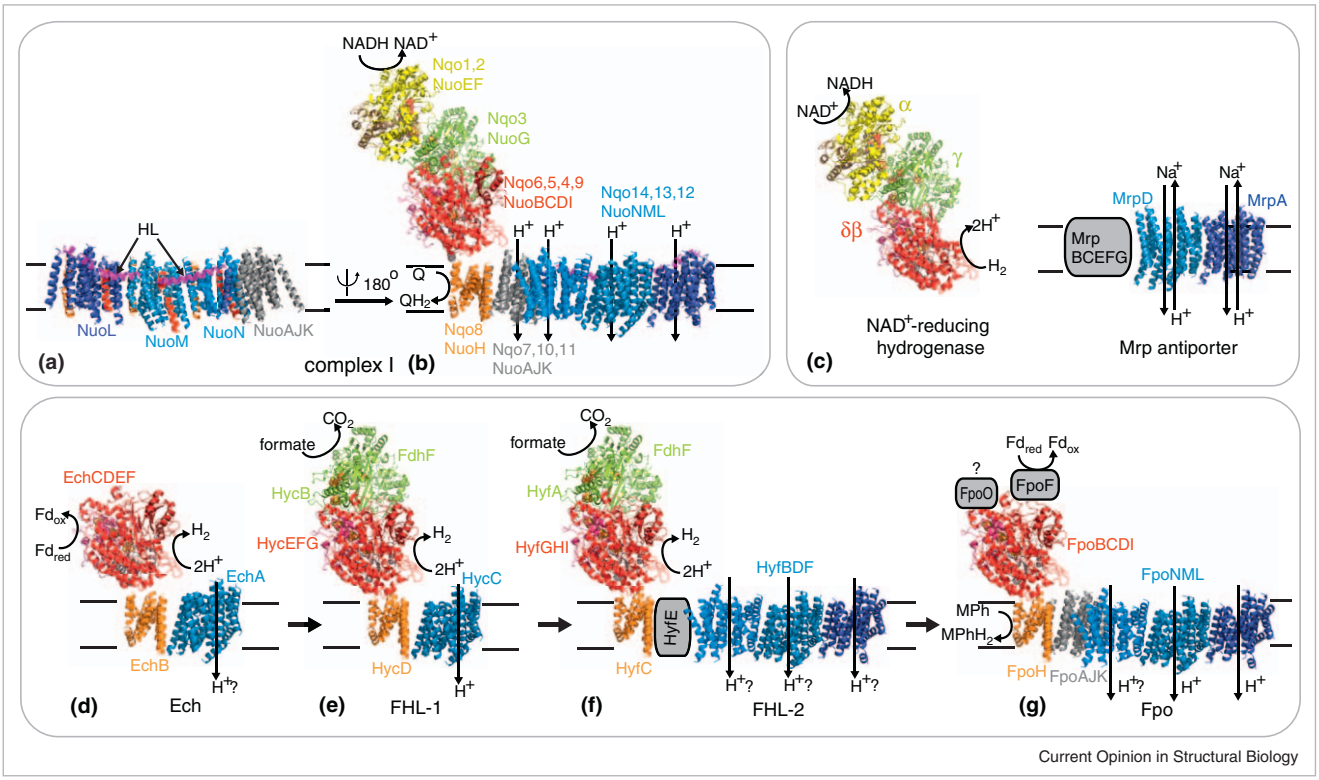
Structures of the hydrophilic domain. **(a)** Side view [25]. Each subunit is coloured differently; FMN is shown as magenta spheres, metal sites as red spheres for Fe atoms and yellow spheres for S atoms. The likely quinone-binding site is indicated (Q). **(b)** Arrangement of redox cofactors in complex I. Cluster N3, NADH-binding site and FMN are in subunit Nqo1; N1a in Nqo2; N1b, N4, N5 and N7 in Nqo3; N6a/b in Nqo9 and N2 in Nqo6. Two electrons are donated from NADH to FMN as a hydride and then transferred one by one through a chain of Fe–S clusters (blue arrows), arranged within 14 Å edge-to-edge of each other (maximal functionally relevant distance [59]). Cluster N2 donates electrons to quinone in the membrane domain. Out-of-chain cluster N7 appears to be an evolutionary remnant and cluster N1a may prevent excessive ROS production by temporarily storing electrons (green arrow) [8,25]. Distances between redox centres are shown in angstroms both centre-to-centre and edge-to-edge (in brackets). The position of NADH is from PDB 3IAM [27••]. **(c)** Conformational changes upon reduction by NADH [27••]. The structure is coloured according to Cα deviations upon reduction, increasing from blue to green to yellow. Higher deviations near the nucleotide-binding site (top, NADH in magenta) and at the interface with the membrane domain (bottom) are evident. Helices H1/H2 from subunit Nqo6 and the four-helix bundle (4HB) from Nqo4 are indicated, with arrows showing their movement upon reduction. **(d)** Environment of cluster N2 [27••]. Left, oxidized domain; middle, fully reduced domain; right, partially reduced domain (cluster N2 reduced but most other clusters oxidized). Disconnection of one of the tandem cysteines (C45/C46) upon reduction is evident from $2F_o - F_c$ electron density, contoured at 1σ. Prefixes before residue names indicate the Nqo subunit number.

hydrogenase (Figure 2d). This evolved further by including additional electron-input (Figure 2e) or proton-translocating subunits (Figure 2f) and adapting for the higher energy flow through the enzyme to the membrane-embedded substrate (Figure 2g) [43,44] (not necessarily in that order, details will be discussed elsewhere). Analysis suggests that the coupling mechanism is similar throughout and originated once during the evolution, together with subunit NuoH, since this subunit is present in all related proton-translocating oxidoreductases and contains many conserved from hydrogenases to complex I charged residues in loops forming the interface with the peripheral domain.

Comparison with mitochondrial enzyme

The best 3D EM reconstructions of complex I from *Yarrowia lipolytica* (16.5 Å resolution), bovine (27 Å) and the thermophile *Aquifex aeolicus* (45 Å) have been

Figure 2

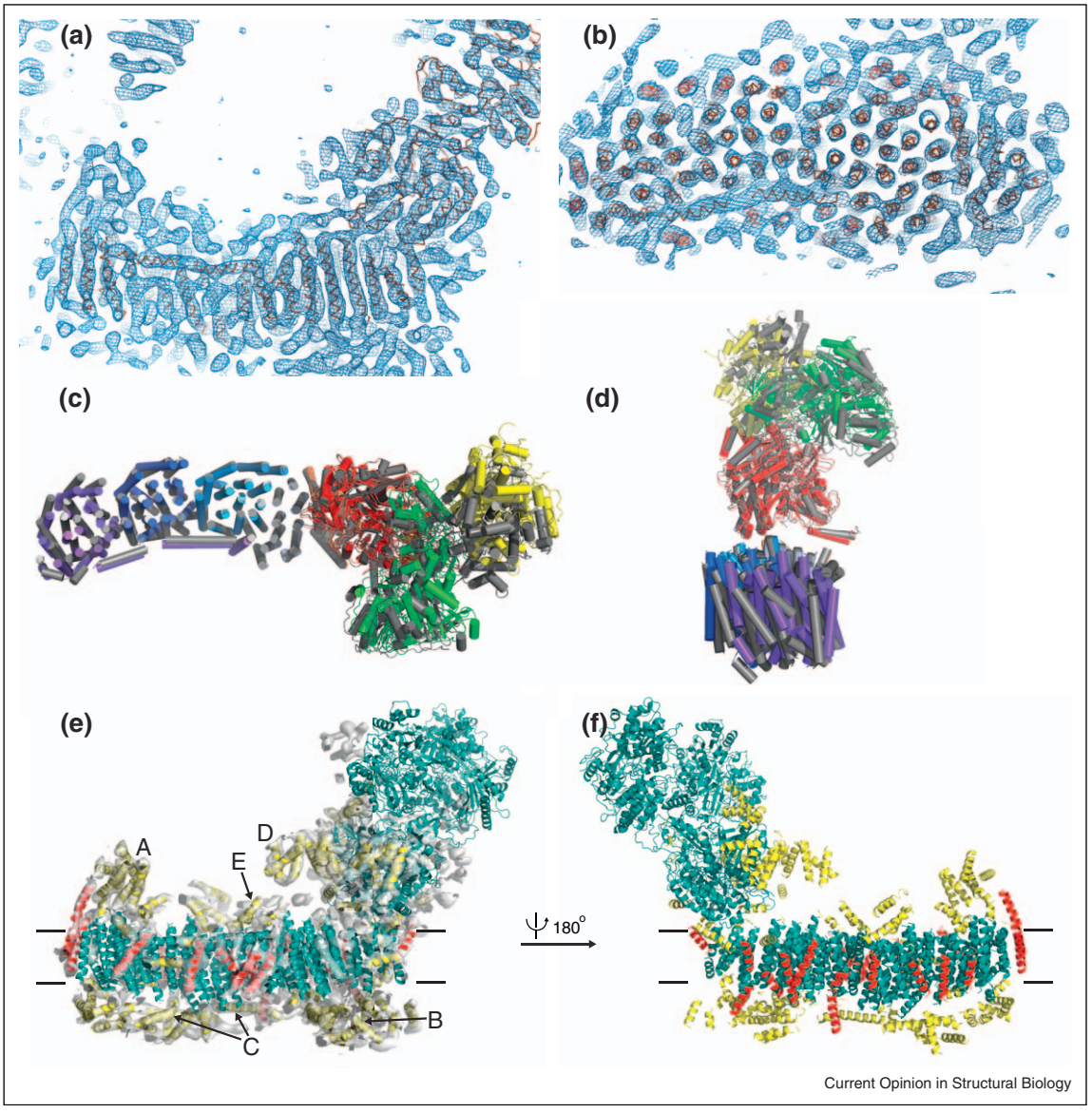


Structure and evolution of complex I. **(a)** Structure of the membrane domain of *E. coli* complex I [14••]. Helix HL is in magenta and discontinuous helices are in red (those contacting HL) or yellow. **(b)** Structure of the entire *T. thermophilus* complex I [14••], with evolutionary modules colour-coded (the same coding is used throughout): yellow and green, N module (NuoEFG) [6]; red, Q module (NuoBCDI); orange, NuoH-like subunits; blue, antiporter-like subunits of P module and grey, adapter subunits connecting Q and P modules. **(c)** Two separate origins of complex I: closely related protein complexes that have either only oxidoreductase activity, bidirectional NAD^+-reducing NiFe hydrogenase (homologous to subunits NuoEFGBD) [60], or transport activity, Mrp antiporters (NuoLMNK; MrpC is homologous to NuoK) [42]. **(d)–(g)** Evolution of complexity, from simplest known complex I-related proton-pumping oxidoreductase, Ech hydrogenase from *Methanosarcina barkeri* (NuoBCDIHM) (d), to formate hydrogen lyase 1 (FHL-1) of *E. coli* (NuoGBCDIHM) (e), to FHL-2 of *E. coli* (NuoGBCDIHKLMN; C-terminus of HyfE is homologous to NuoK) (f) [60] and to 11 subunit archeal homologue, Fpo complex from *Methanosarcina mazei* (NuoBCDIHAJKLMN) (g) [61]. NDH-1 complex from chloroplasts and cyanobacteria also contains 11 core subunits, lacking N module but with many additional subunits present, although the identity of electron donor is unclear [62,63]. Names of subunits constituting the complexes are indicated. Subunits unrelated to complex I are shown as grey rectangles. Question marks indicate characteristics that have not been unambiguously established experimentally, such as proton-pumping of Ech and FHL-2, H^+/e^- stoichiometry of Fpo, as well as association of subunit FpoO with the complex. *Abbreviations*: MPh: methanophenazine; Fd_{red} and Fd_{ox}: reduced and oxidized ferredoxin; Q: ubiquinone or menaquinone.

obtained with deep stain technique [45,46•]. However, the comparison with our X-ray structure (Fig. S7 in [14••]) clearly demonstrated that the hand of these reconstructions has been inverted, producing a mirror-image [46•]. As a result, previous fits of the hydrophilic domain X-ray structure into these EM reconstructions could not have been correct. Fit number 2 out of 5 in [47] slightly resembles the actual arrangement by chance, leaving a big sub-domain empty. It was dismissed when the *A. aeolicus* reconstruction became available [46•], but, surprisingly, was quoted again in the recent X-ray analysis paper [48••]. Since the choice of hand for our earlier cryo-EM reconstruction of *E. coli* enzyme was based on previous models from the same group [45], the hand in our EM reconstruction was also reversed [37]. Hopefully, corrected EM reconstructions of at least the bovine enzyme, for which there is no X-ray data, will become available.

The first X-ray analysis (at 6.3 Å resolution) of mitochondrial enzyme, from yeast *Y. lipolytica*, was published recently [48••], shortly after the bacterial enzyme structure [14••]. The arrangement of Fe–S clusters was shown to be similar to that in the bacterial enzyme, but the individual subunits were not identified [48••]. Comparison of the published electron density map of *Y. lipolytica* complex I (kindly provided by the authors) to our structure of *T. thermophilus* complex I is very informative. Most α-helices are clearly resolved in the map as tubular densities, which allowed us to fit bacterial complex using CHIMERA [49]. Remarkably, after the refinement of individual subunits as rigid bodies, nearly every

Figure 3



Comparison of bacterial and mitochondrial complexes I. **(a)** and **(b)** Fit of *T. thermophilus* complex I [14••], with individual subunits (shown as brown ribbons) refined as rigid bodies into the electron density of *Y. lipolytica* enzyme. The experimental density calculated to 6.3 Å with phases determined from TaBr derivative followed by density modification [48••] is shown at 1σ. (a) Side view, with TM and HL helices visible. (b) Top view, with TM helices normal to the plane. Extra TM helices from accessory subunits, at the periphery, are in red. **(c)** and **(d)** Re-arrangements of core subunits. Overlay of bacterial structure (grey) and mitochondrial model (coloured similarly as in Figure 2). (c) Top view from the matrix side. (d) Front view, from subunit NuoL/ND5. **(e)** and **(f)** Additional helices from accessory subunits, with TM helices in red and others in yellow. Core subunits are green. The density for extra subunits (grey) is shown in (e). Additional domains, mentioned in the text, are indicated (A–E).

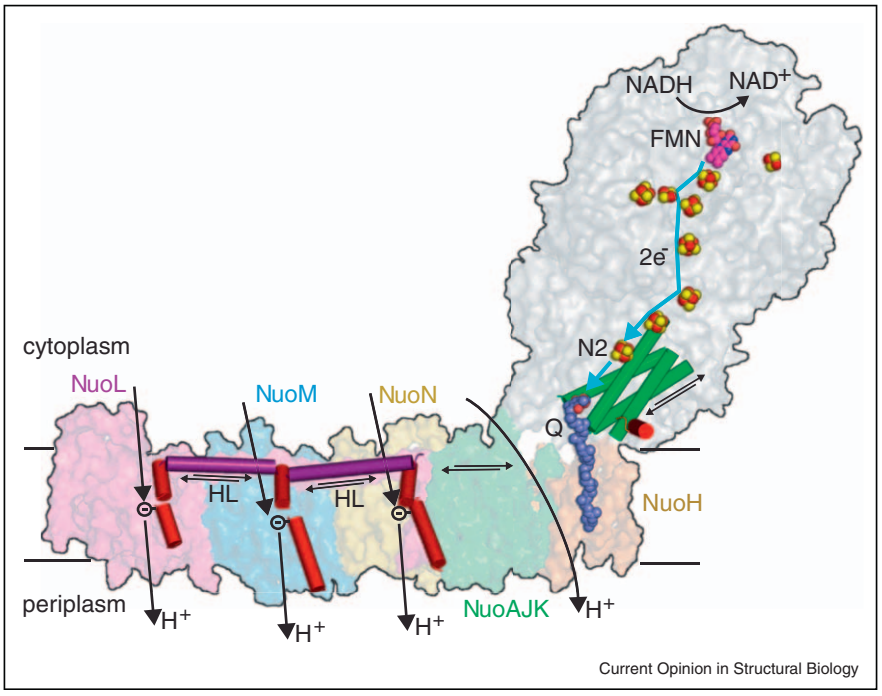
single helix of the bacterial enzyme fits perfectly into the electron density of the mitochondrial enzyme (Figure 3a and b). It appears that upon billions of years of evolution the structures of individual subunits have been fully preserved, and only slight relative rearrangement of subunits occurred. The junction of the two main domains is mostly unchanged, whilst dehydrogenase domain subunits NuoEFG/Nqo1,2,3 (51,24,75 kDa in bovine nomenclature) are rotated about 10° around an axis centred at cluster N6a and roughly parallel to the long axis of the membrane domain (Figure 3c and d). There is also a screw-like rearrangement of antiporter-like subunits propagating from NuoN/Nqo14/ND2 towards NuoL/Nqo12/ND5, leading to a rotation of ND5 of about 10° around the long axis of the membrane domain (Figure 3c and d).

Crucially, mechanistically important elements are conserved. The long amphipathic helix HL has the same length (estimates of shorter extent in [48••] were presumably due to a few intermittent areas of lower density), and flanking NuoL/Nqo12/ND5 helices TM15 and TM16 are found at the same positions in prokaryotic and eukaryotic complexes (Figure 3a). Furthermore, all six discontinuous TM helices (two in each antiporter-like subunit) are also discontinuous and occupy the same positions.

Evolution of the mitochondrial enzyme resulted in the association of up to 31 (bovine [13]) or 26 (*Y. lipolytica* [50]) supernumerary or accessory subunits. At least three of them were likely acquired before the symbiotic event which created mitochondria [39] and the rest during the evolution of eukaryotes [51]. Their putative roles include assistance in the assembly, stability and regulation of the complex [50,51]. For the *Y. lipolytica* enzyme, 15 extra TM helices are predicted (eight of them from single transmembrane domain (STMD) proteins [50]) and about eight supernumerary subunits are likely to be associated with the hydrophilic domain [52]. Comparison of the bacterial structure with *Y. lipolytica* map allows clear visualization of additional electron density corresponding to the supernumerary subunits (Figure 3b and e). We built helices into this extra density using the 'find_helices_strands' module in Phenix [53] (Supplementary Movie 2). Sixteen additional TM helices are evenly distributed, mostly as single isolated TM helices, around core membrane subunits (Figure 3e and f). Most are tilted, similar to the arrangement of accessory subunits in cytochrome *c* oxidase, in agreement with their stabilizing role [50]. This brings the total number of TM helices observed in *Y. lipolytica* to about 79, consistent with predictions [52]. Other prominent additions to the membrane domain are: at the matrix side, a six-helix bindle at the tip, near subunit ND5 (A in Figure 3e); at the intermembrane side, a globular domain near NuoH/Nqo8/ND1 subunit (B) and several long helices (C) running along the length of the domain. The latter may improve stability and/or communication within the complex.

In the peripheral domain additional subunits are observed only near subunits at the connection of the peripheral and membrane domains (NuoBCDI/Nqo4-6,9), probably providing additional stability to this fragile junction (Figure 3e and f). A prominent six-helix protrusion emanating from near NuoC/Nqo5/30 kDa (D in Figure 3e) likely contacts additional subunit(s) near NuoN/Nqo14/ND2 (E), again possibly improving stability. Subunit

Figure 4



Proposed mechanism of complex I. NADH, via FMN (magenta) and the chain of Fe–S clusters (red and yellow spheres) reduces quinone (dark blue). Electron transfer is coupled to conformational changes (indicated by arrows) in the hydrophilic domain, observed (Figure 1c) for NuoD/Nqo4 four-helix bundle (green cylinders) and NuoB/Nqo6 helix H1 (red). These changes are transmitted, via subunits NuoHAJK, to the amphipathic helix HL (magenta), which tilts the three discontinuous helices (red) in antiporter-like subunits, changing the conformation of ionizable residues inside the respective proton channels, resulting in the translocation of three protons. The forth proton is translocated at the interface of the two main domains. The hydrophilic domain surface is in grey, membrane domain subunits coloured as indicated.

Nqo15 is not present in mitochondrial enzymes and does not appear to have been replaced by any other protein mass.

Thus, supernumerary subunits do not interfere with the fold of core subunits, but instead form an outer shell, consistent with their proposed roles in facilitating stability and assembly of the complex [39,50,51]. The remarkable conservation of the structure of the core subunits from bacteria to mitochondria strengthens the idea that the mechanism of complex I is the same throughout all species.

Mechanism of complex I

The overall architecture of complex I has strong implications for the coupling mechanism. The NuoDB/Nqo4,6 helices, which shift upon reduction (Figure 1b and c), are well positioned to transmit conformational changes to subunits NuoAHJK/Nqo7,8,10,11, around the putative Q-site (Figure 4). Indeed, cross-links between NuoA/Nqo7 and NuoJ/Nqo10 change upon reduction [23]. Furthermore, conformational changes may be aided by the unexpected, but necessary, quinone movement to and from cluster N2 upon binding, reduction and release, in agreement with the suggestion that most of energy from NADH is released only upon delivery of its second electron to quinone [54•].

A possible mechanism (Figure 4) is that conformational changes in subunits NuoAHJK/Nqo7,8,10,11 affect the nearby C-terminal helix of NuoL/Nqo12, leading to a piston-like motion of helix HL along the membrane domain. This movement will synchronously tilt the three discontinuous helices in antiporter-like subunits that are in direct contact with HL. Some of known conserved and essential charged residues are likely to be found in these discontinuous helices, exposed inside putative channels. Hence, their movement, driven at least in part by helix HL, will result in proton translocation. In total, three protons would be translocated (one per each antiporter-like subunit). The fourth proton per cycle, to account for known stoichiometry, is probably translocated, in a conformation-driven manner, at the interface of the two main domains via subunits NuoAJK/Nqo7,10,11, which also contain essential charged residues within predicted TM helices [55,56]. Since cluster N2 is elevated over the membrane it is unlikely to participate directly in proton translocation as suggested in [27••]. Rather, disconnection of the tandem cysteines may promote conformational changes (and possibly also protonation of quinone, as discussed [27••]).

Recent studies on *E. coli* mutants with C-terminally truncated versions of NuoL confirmed the essential coupling role of helix HL [57•]. Very qualitative measurements suggested that about two protons per cycle are still translocated in mutants [57•]. If this value is correct it would imply that subunit NuoN is less reliant on HL for coupling, consistent with its position right next to subunits NuoAJK. The authors proposed an alternative explanation based on the assumption that subunit NuoN does not translocate protons [57•,58], which is not likely in our opinion (manuscript in preparation).

Concluding remarks

Recent structural data suggest that the overall design of complex I resembles a steam engine, with helix HL playing the role of a coupling rod, which drives, instead of wheels, a set of helices. Several related protein families are likely to employ similar mechanisms. This exciting finding adds to the variety of biological molecular machines, including the turbine-like F-ATPase that resemble human creations (or vice versa). The molecular details remain to be established, which will require atomic structures for the membrane domain and the entire complex I.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.sbi. 2011. 07.002.

Acknowledgements

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Galkin AS, Grivennikova VG, Vinogradov AD: **$H^+/2e^-$ stoichiometry in NADH–quinone reductase reactions catalyzed by bovine heart submitochondrial particles**. *FEBS Lett* 1999, **451**:157-161.

2. Galkin A, Drose S, Brandt U: **The proton pumping stoichiometry of purified mitochondrial complex I reconstituted into proteoliposomes**. *Biochim Biophys Acta* 2006, **1757**:1575-1581.

3. Wikstrom M: **Two protons are pumped from the mitochondrial matrix per electron transferred between NADH and ubiquinone**. *FEBS Lett* 1984, **169**:300-304.

4. Walker JE: **The NADH–ubiquinone oxidoreductase (complex I) of respiratory chains**. *Q Rev Biophys* 1992, **25**:253-324.

5. Yagi T, Matsuno-Yagi A: **The proton-translocating NADH–quinone oxidoreductase in the respiratory chain: the secret unlocked**. *Biochemistry* 2003, **42**:2266-2274.

6. Brandt U: **Energy converting NADH:quinone oxidoreductase (complex I)**. *Annu Rev Biochem* 2006, **75**:69-92.

7. Ohnishi T: **Iron–sulfur clusters/semiquinones in complex I**. *Biochim Biophys Acta* 1998, **1364**:186-206.

8. Sazanov LA: **Respiratory complex I: mechanistic and structural insights provided by the crystal structure of the hydrophilic domain**. *Biochemistry* 2007, **46**:2275-2288.

9. Hirst J: **Towards the molecular mechanism of respiratory complex I**. *Biochem J* 2010, **425**:327-339.

10. Schapira AH: **Human complex I defects in neurodegenerative diseases**. *Biochim Biophys Acta* 1998, **1364**:261-270.

11. Dawson TM, Dawson VL: **Molecular pathways of neurodegeneration in Parkinson's disease**. *Science* 2003, **302**:819-822.

12. Balaban RS, Nemoto S, Finkel T: **Mitochondria, oxidants, and aging**. *Cell* 2005, **120**:483-495.

13. Carroll J, Fearnley IM, Skehel JM, Shannon RJ, Hirst J, Walker JE: **Bovine complex I is a complex of 45 different subunits**. *J Biol Chem* 2006, **281**:32724-32727.

14. •• Efremov RG, Baradaran R, Sazanov LA: **The architecture of respiratory complex I**. *Nature* 2010, **465**:441-445.
First structures of the membrane domain of *E. coli* complex I (at 3.9 Å resolution) and the entire *T. thermophilus* enzyme (4.5 Å resolution). The membrane domain was resolved at the α-helical level in both cases and the arrangement of subunits was determined, with many functional implications. Unusual coupling rod-like helix HL was shown to originate from subunit NuoL.

15. Friedrich T: **Complex I: a chimaera of a redox and conformation-driven proton pump?** *J Bioenerg Biomembr* 2001, **33**:169-177.

16. Watt IN, Montgomery MG, Runswick MJ, Leslie AG, Walker JE: **Bioenergetic cost of making an adenosine triphosphate molecule in animal mitochondria**. *Proc Natl Acad Sci U S A* 2010, **107**:16823-16827.

17. Wikstrom M, Verkhovsky MI: **Towards the mechanism of proton pumping by the haem–copper oxidases**. *Biochim Biophys Acta* 2006, **1757**:1047-1051.

18. Fearnley IM, Walker JE: **Conservation of sequences of subunits of mitochondrial complex I and their relationships with other proteins**. *Biochim Biophys Acta* 1992, **1140**:105-134.

19. Mathiesen C, Hagerhall C: **Transmembrane topology of the NuoL, M and N subunits of NADH:quinone oxidoreductase and their homologues among membrane-bound hydrogenases and bona fide antiporters**. *Biochim Biophys Acta* 2002, **1556**:121-132.

20. Baranova EA, Morgan DJ, Sazanov LA: **Single particle analysis confirms distal location of subunits NuoL and NuoM in *Escherichia coli* complex I**. *J Struct Biol* 2007, **159**:238-242.

21. Belogrudov G, Hatefi Y: **Catalytic sector of complex I (NADH:ubiquinone oxidoreductase): subunit stoichiometry and substrate-induced conformation changes**. *Biochemistry* 1994, **33**:4571-4576.

22. Mamedova AA, Holt PJ, Carroll J, Sazanov LA: **Substrate-induced conformational change in bacterial complex I**. *J Biol Chem* 2004, **279**:23830-23836.

23. Berrisford JM, Thompson CJ, Sazanov LA: **Chemical and NADH-induced, ROS-dependent, cross-linking between subunits of complex I from *Escherichia coli* and *Thermus thermophilus***. *Biochemistry* 2008, **47**:10262-10270.

24. Yamaguchi M, Belogrudov GI, Hatefi Y: **Mitochondrial NADH–ubiquinone oxidoreductase (complex I). Effect of substrates on the fragmentation of subunits by trypsin**. *J Biol Chem* 1998, **273**:8094-8098.

25. Sazanov LA, Hinchliffe P: **Structure of the hydrophilic domain of respiratory complex I from *Thermus thermophilus***. *Science* 2006, **311**:1430-1436.

26. Hinchliffe P, Sazanov LA: **Organization of iron–sulfur clusters in respiratory complex I**. *Science* 2005, **309**:771-774.

27. •• Berrisford JM, Sazanov LA: **Structural basis for the mechanism of respiratory complex I**. *J Biol Chem* 2009, **284**:29773-29783.
First structures of the reduced hydrophilic domain of complex I are showing how NADH interacts with the enzyme and how the conformation of the domain changes upon reduction. Intriguing disconnection of tandem cysteines from Fe–S cluster N2 discovered.

28. Hinchliffe P, Carroll J, Sazanov LA: **Identification of a novel subunit of respiratory complex I from *Thermus thermophilus***. *Biochemistry* 2006, **45**:4413-4420.

29. Kussmaul L, Hirst J: **The mechanism of superoxide production by NADH:ubiquinone oxidoreductase (complex I) from bovine heart mitochondria**. *Proc Natl Acad Sci U S A* 2006, **103**:7607-7612.

30. Kudin AP, Bimpong-Buta NY, Vielhaber S, Elger CE, Kunz WS: **Characterization of superoxide-producing sites in isolated brain mitochondria**. *J Biol Chem* 2004, **279**:4127-4135.

31. Grivennikova VG, Vinogradov AD: **Generation of superoxide by the mitochondrial complex I**. *Biochim Biophys Acta* 2006, **1757**:553-561.

32. Galkin A, Brandt U: **Superoxide radical formation by pure complex I (NADH:ubiquinone oxidoreductase) from *Yarrowia lipolytica***. *J Biol Chem* 2005, **280**:30129-30135.

33. Torres-Bacete J, Sinha PK, Castro-Guerrero N, Matsuno-Yagi A, Yagi T: **Features of subunit NuoM (ND4) in *Escherichia coli* NDH-1: topology and implication of conserved Glu144 for coupling site 1**. *J Biol Chem* 2009, **284**:33062-33069.

34. Screpanti E, Hunte C: **Discontinuous membrane helices in transport proteins and their correlation with function**. *J Struct Biol* 2007, **159**:261-267.

35. Vik SB: **The transmembrane helices of the L, M, and N subunits of complex I from *E. coli* can be assigned on the basis of conservation and hydrophobic moment analysis**. *FEBS Lett* 2011. [epub].

36. Sazanov LA, Carroll J, Holt P, Toime L, Fearnley IM: **A role for native lipids in the stabilization and two-dimensional crystallization of the *Escherichia coli* NADH–ubiquinone oxidoreductase (complex I)**. *J Biol Chem* 2003, **278**:19483-19491.

37. Morgan DJ, Sazanov LA: **Three-dimensional structure of respiratory complex I from *Escherichia coli* in ice in the presence of nucleotides**. *Biochim Biophys Acta* 2008, **1777**:711-718.

38. Peng G, Fritzsch G, Zickermann V, Schagger H, Mentele R, Lottspeich F, Bostina M, Radermacher M, Huber R, Stetter KO *et al.*: **Isolation, characterization and electron microscopic single particle analysis of the NADH:ubiquinone oxidoreductase (complex I) from the hyperthermophilic eubacterium *Aquifex aeolicus***. *Biochemistry* 2003, **42**:3032-3039.

39. Yip CY, Harbour ME, Jayawardena K, Fearnley IM, Sazanov LA: **Evolution of respiratory complex I: ''supernumerary'' subunits are present in the alpha-proteobacterial enzyme**. *J Biol Chem* 2011, **286**:5023-5033.

40. Guenebaut V, Schlitt A, Weiss H, Leonard K, Friedrich T: **Consistent structure between bacterial and mitochondrial NADH:ubiquinone oxidoreductase (complex I)**. *J Mol Biol* 1998, **276**:105-112.

41. Friedrich T, Scheide D: **The respiratory complex I of bacteria, archaea and eukarya and its module common with membrane-bound multisubunit hydrogenases**. *FEBS Lett* 2000, **479**:1-5.

42. Mathiesen C, Hagerhall C: **The 'antiporter module' of respiratory chain complex I includes the MrpC/NuoK subunit — a revision of the modular evolution scheme**. *FEBS Lett* 2003, **549**:7-13.

43. Hedderich R: **Energy-converting [NiFe] hydrogenases from archaea and extremophiles: ancestors of complex I**. *J Bioenerg Biomembr* 2004, **36**:65-75.

44. Fontecilla-Camps JC, Volbeda A, Cavazza C, Nicolet Y: **Structure/function relationships of [NiFe]- and [FeFe]-hydrogenases**. *Chem Rev* 2007, **107**:4273-4303.

45. Radermacher M, Ruiz T, Clason T, Benjamin S, Brandt U, Zickermann V: **The three-dimensional structure of complex I from *Yarrowia lipolytica*: a highly dynamic enzyme**. *J Struct Biol* 2006, **154**:269-279.

46. • Clason T, Ruiz T, Schagger H, Peng G, Zickermann V, Brandt U, Michel H, Radermacher M: **The structure of eukaryotic and prokaryotic complex I**. *J Struct Biol* 2010, **169**:81-88.

Current best 3D EM reconstructions for bovine, *Y. lipolytica* and *A. aeolicus* enzymes. However, the hand of the reconstructions is inverted, resulting in mirror-image density.

47. Clason T, Zickermann V, Ruiz T, Brandt U, Radermacher M: **Direct localization of the 51 and 24 kDa subunits of mitochondrial complex I by three-dimensional difference imaging**. *J Struct Biol* 2007, **159**:433-442.

48. •• Hunte C, Zickermann V, Brandt U: **Functional modules and structural basis of conformational coupling in mitochondrial complex I**. *Science* 2010, **329**:448-451.
First X-ray analysis of mitochondrial complex I, from *Y. lipolytica*, at 6.3 Å resolution. Arrangement of Fe–S clusters is shown to be similar to that in the bacterial enzyme. A distant position of cluster N2 from the membrane is noted, and ubiquinone is suggested to move towards N2 over a hydrophobic ramp. Helix HL observed, but individual subunits in the membrane domain have not been identified.

49. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE: **UCSF chimera — a visualization system for exploratory research and analysis**. *J Comp Chem* 2004, **25**:1605-1612.

50. Zickermann V, Angerer H, Ding MG, Nubel E, Brandt U: **Small single transmembrane domain (STMD) proteins organize the hydrophobic subunits of large membrane protein complexes**. *FEBS Lett* 2010, **584**:2516-2525.

51. Gabaldon T, Rainey D, Huynen MA: **Tracing the evolution of a large protein complex in the eukaryotes, NADH:ubiquinone oxidoreductase (complex I)**. *J Mol Biol* 2005, **348**:857-870.

52. Zickermann V, Kerscher S, Zwicker K, Tocilescu MA, Radermacher M, Brandt U: **Architecture of complex I and its implications for electron transfer and proton pumping**. *Biochim Biophys Acta* 2009, **1787**:574-583.

53. Adams PD, Grosse-Kunstleve RW, Hung LW, Ioerger TR, McCoy AJ, Moriarty NW, Read RJ, Sacchettini JC, Sauter NK, Terwilliger TC: **PHENIX: building new software for automated crystallographic structure determination**. *Acta Crystallogr D* 2002, **58**:1948-1954.

54. • Verkhovskaya ML, Belevich N, Euro L, Wikstrom M, Verkhovsky MI: **Real-time electron transfer in respiratory complex I**. *Proc Natl Acad Sci U S A* 2008, **105**:3763-3767.
Electron transfer in complex I from *E. coli* was investigated by an ultrafast freeze-quench approach. Data suggest that transfer of the first electron from NADH to ubiquinone is not associated with any drop of energy, whilst the transfer of the second electron occurs across a large potential drop that drives translocation of all four protons.

55. Kao MC, Nakamaru-Ogiso E, Matsuno-Yagi A, Yagi T: **Characterization of the membrane domain subunit NuoK (ND4L) of the NADH–quinone oxidoreductase from *Escherichia coli***. *Biochemistry* 2005, **44**:9545-9554.

56. Kervinen M, Patsi J, Finel M, Hassinen IE: **A pair of membrane-embedded acidic residues in the NuoK subunit of *Escherichia coli* NDH-1, a counterpart of the ND4L subunit of the mitochondrial complex I, are required for high ubiquinone reductase activity**. *Biochemistry* 2004, **43**:773-781.

57. • Steimle S, Bajzath C, Dorner K, Schulte M, Bothe V, Friedrich T: **The role of subunit NuoL for proton translocation by the respiratory complex I**. *Biochemistry* 2011. [epub].
First mutagenesis studies involving helix HL, using C-terminally truncated versions of subunit NuoL in *E. coli*. The coupling role of helix HL is confirmed, although data on proton-pumping stoichiometry are very qualitative.

58. Ohnishi ST, Salerno JC, Ohnishi T: **Possible roles of two quinone molecules in direct and indirect proton pumps of bovine heart NADH–quinone oxidoreductase (complex I)**. *Biochim Biophys Acta* 2010, **1797**:1891-1893.

59. Page CC, Moser CC, Chen X, Dutton PL: **Natural engineering principles of electron tunnelling in biological oxidation–reduction**. *Nature* 1999, **402**:47-52.

60. Vignais PM, Billoud B: **Occurrence, classification, and biological function of hydrogenases: an overview**. *Chem Rev* 2007, **107**:4206-4272.

61. Baumer S, Ide T, Jacobi C, Johann A, Gottschalk G, Deppenmeier U: **The F420H2 dehydrogenase from *Methanosarcina mazei* is a Redox-driven proton pump closely related to NADH dehydrogenases**. *J Biol Chem* 2000, **275**:17968-17973.

62. Sazanov LA, Burrows PA, Nixon PJ: **The plastid ndh genes code for an NADH-specific dehydrogenase: isolation of a complex I analogue from pea thylakoid membranes**. *Proc Natl Acad Sci U S A* 1998, **95**:1319-1324.

63. Peng L, Yamamoto H, Shikanai T: **Structure and biogenesis of the chloroplast NAD(P)H dehydrogenase complex**. *Biochim Biophys Acta* 2010, **1807**:945-953.